

FIRST RESULTS FROM EXPANDED NEWBORN SCREENING IN THE SLOVAK REPUBLIC

PRVÉ VÝSLEDKY ROZŠÍŘENÉHO NOVORODENECKÉHO SKRÍNINGU V SLOVENSKEJ REPUBLIKE

Original research article

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Abstract A one-and-a-half year experience with expanded newborn screening (ENBS) in Slovakia provided by means of tandem mass spectrometry (LC-MS/MS) is presented.

Method ENBS was realised from dry blood samples of the regular NBS in National Screening Centre SK. The LC-MS/MS software used allowed evaluating even 73 analytes in MRM mode after cut-off limits in 99 percentile of daily values. The regular ENBS was oriented to 10 inherited metabolic disorders (IMDs), organic acids and carnitine defects (PKU/HPA, MSUD, GAI, IVA, MCAD, LCHAD, SCAD, CPT I, CPT II, CACT). Except these, many other variations of results were registered in perimeter of the method. They were individually evaluated until the confirmation or exclusion of the suspected diagnosis.

Results During the one-and-a-half years, 82 892 newborns were evaluated by ENBS and 34 positive cases of IMDs were detected and confirmed, which represents a screening prevalence of 1:2438 newborns. Out of these, 24 cases were from regular ENBS (PKU/HPA, MSUD, MCAD) with an incidence of 1:3 454, and 10 additional cases at the periphery of LC-MS/MS evaluation with a prevalence of 1:8 286.

Conclusion Preliminary experience and results with ENBS confirmed its high effectiveness not only in 10 IMDs included in regular ENBS, but also at perimeter of LC-MS/MS method, in which it is possible to detect additional IMDs not included in regular spectrum of ENBS. This group comprised 29% of all cases. In general, the prevalence is comparable with PKU and/or CAH.

Slovak abstract Autori prezentujú jeden a pol ročné výsledky rozšíreného novorodeneckého skrínungu dedičných metabolických porúch (ENBS) pomocou vyšetrenia tandemovou hmotnostnou spektrometriou (LC-MS/MS) na Slovensku.

Metódy Rozšírený novorodenecký skrínung dedičných metabolických porúch bol realizovaný zo vzoriek naberaných u novorodencov pre štandardný skrínung. Tandemová hmotnostná spektrometria LC MS/MS vo svojom špeciálne upravenom software zachytáva a vyhodnocuje až 73 analytov v MRM móde, podľa denne kalkulovaných cut-off limitov v 99 percentile priemerov hodnôt. Pravidelný ENBS bol zameraný na desať dedičných metabolických porúch (DMP) v oblasti organických acidúrií, mastných kyselín a porúch karnitínu (PKU, MSUD, GAI, IVA, MCAD, LCHAD, SCAD, CPT I, CPT II, CACT). Okrem toho bol v „periférii“ vyšetrenia zaznamenávaný celý rad porúch, ktoré boli vyhodnotené individuálne až do definitívneho potvrdenia/vylúčenia diagnózy.

Výsledky V ENBS v priebehu 1,5 roka bolo vyšetrených 82 893 novorodencov a zachytených bolo 34 prípadov DMP, čo je prevalencia 1 : 2 438 novorodencov. Z nich 24 boli diagnózy v pravidelnom ENBS (PKU/HPA, MSUD, MCAD) - incidencia 1 : 3 454, a 10 v „periférii“ LC-MS/MS hodnotenia – incidencia 1 : 8 286.

Záver Už predbežné výsledky potvrdzujú vysokú efektivitu ENBS, kedy skrínungová prevalencia desať DMP v pravidelnom skrínungu spolu s periférnymi zachytmi LC-MS/MS dosahuje vysokú prevalenciu. Prípady DMP detegované nad pravidelný ENBS („periférne“) predstavujú až 29 % všetkých zachytov a ich prevalencia je porovnateľná s incidenciou PKU a CAH.

Keywords extended newborn screening, tandem mass spectrometry

Kľúčové slová rozšírený novorodenecký skrínung, tandemová hmotnostná spektrometria

INTRODUCTION

Newborn screening (NBS), which was started in the mid-20th century, is the one of first preventive measures investigating inherited metabolic disorders (IMDs) early after birth, in asymptomatic stage, thus preventing irreversible health damage in positive cases. The spectrum of disorders covered

by NBS has been steadily increasing, according to the development of screening laboratory methods, confirmatory and treatment possibilities (Zabransky 2002). From a recent point of view, the NBS represents the typical model of comprehensive care for rare disorders also, which is one of the

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crucial targets in health work packages of European Union (The European Union Committee of Experts on Rare Diseases - EUCERD) (Loeber et al. 2012, Burgard et al. 2012). The expanded programme of NBS (expanded newborn screening (ENBS)) considers the Wilson–Jungner criteria (W-Jc) of suitability for screening (Wilson and Jungner, 1968). At the end of 20th century, the introduction of tandem mass spectrometry (LC-MS/MS) into NBS significantly extended the spectrum of detectable IMDs in ENBS (Pollitt 2007, Wilcken et al. 2009). Several countries in the developed world expanded their spectrum of disorders detectable by NBS, taking into account W-Jc, economic factors and other criteria (Pollitt 2007, Wilcken 2011).

NBS in the Slovak Republic started in the national Newborn Screening Center (NBSC) in 1985 for congenital hypothyroidism (CH) and phenylketonuria/hyperphenylalaninemia (PKU/HPA), followed by congenital adrenal hyperplasia (CAH), and cystic fibrosis (CF). Results of this part of NBS were published elsewhere (Dluholucký, Knapková and Záhorcová 2013).

In spring 2012, the LC-MS/MS device was obtained in NSC, and during 1 June–31 December 2012, the pilot study of ENBS was performed. The results of the ENBS were accepted positively. Thus from 1 January 2013, ENBS is performed in Slovakia. This paper presents the first results of ENBS in Slovakia.

MATERIAL AND METHOD

Derivatised method of the adjusted dry blood sample (DBS) was analysed on LC-MS/MS method by scanning two ions, characteristic for a given analytical substance (marker), and defined in a multi-reaction monitoring mode. This mode is especially suitable for the detection of polar to middle polar metabolites. In ENBS in Slovakia, attention has been focused on amino acids, fatty acids and acylcarnitines. In one DBS, even as many as 73 analytes were detected and some of them evaluated, using an online system of a special software program. The cut-off limit for each marker was established after

comparison with regional centres of genetics, NBSCs abroad and statistically corrected to 99% percentile value of daily results of analysis. The regular ENBS is focused on 10 IMDs (PKU/HPA, leucinosi, maple syrup urine disease (MSUD), isovaleric acidemia (IVA), glutaric aciduria I, medium chain acyl CoA dehydrogenase deficiency (MCAD), long chain acyl CoA dehydrogenase deficiency (LCHAD), short chain acyl CoA dehydrogenase deficiency (SCAD), carnitin palmitoyl transferase I deficiency (CPT I), carnitin palmitoyl transferase II deficiency (CPT II), carnitin acylcarnitin translocase deficiency (CACT)). These IMDs as well as the algorithm of screening are defined in the guideline of the Slovak Ministry of Health (Professional Guidance of the Ministry of health of the Slovak republic 42/2012). Except these IMDs, in the “periphery” of LC-MS/MS analysis, many deviations were detected. All these potentially positive results at periphery underwent a recall into NSC (in case of persisted positivity). Every case was solved individually in centres for metabolic diseases. Except for these regular investigations, the NBS provided an additional, so-called selective screening, which involves an immediate differential diagnostic procedure in critically newborns and infants admitted in prenatal intensive care unit and neonatal intensive care unit in Slovakia. The results of the selective screening are not included in this paper.

RESULTS

During 2013 (1 January 2013 – 31 December 2013), ENBS was provided in 54 893 newborns (Table 1). Twenty-two positive and confirmed cases of IMDs were detected. From the group of aminoacidopathies, five cases of PKU, four HPA and two cases of suspect thyrosinaemia (extremely high thyrosine – definitively solved in centre for IMDs) were confirmed. Except these, one case of galactosemia was discovered after unusually increased levels of phenylalanine/thyrosine and their ratio. This IMD of saccharide metabolism was captured through amino acid abnormality.

Table 1. Results of expanded newborn screening in NBSC Banská Bystrica (Slovakia) in 2013 (54 893 newborns) (1 January 2013 – 31 December 2013)

Disorder	No. of positive cases	Prevalence
CH	23	1:2 386
CAH	4	1:13 723
CF	5	1:10 978
IMD (tandem mass spectrometry)	22	1:2 495
Out of this PKU/HPA	9	1:6 099
Out of this MCAD	3	1:18 297
Other: galaktosemia (1), hypertyrozinemia (2), maternal CUD (2), propionic acidemia (1), 3-MCCD (2), maternal 3-MCCD (2)	10	1:5 489

CAH congenital adrenal hyperplasia; CF cystic fibrosis; PKU/HPA phenylketonuria/hyperphenylalaninemia; MCAD medium chain acyl CoA dehydrogenase deficiency; CUD carnitine uptake deficiency; 3-MCCD 3-methylcrotonyl CoA carboxylase deficiency

In the group of organic acids, one case of propionic acidemia and two cases of 3-methylcrotonyl coenzyme A carboxylase deficiency (3-MCCD), as well as two cases of maternal 3-MCCD (out of 54 893 newborns) were detected. In the group of free fatty acid oxidation defects, three cases of MCAD, confirmed by PCR, were captured. Also, thanks to the NBS, two cases of maternal carnitine uptake deficiency (CUD) were confirmed; however, the newborns were healthy. In summary, during 2013, 22 cases of IMDs were detected by ENBS; the prevalence of IMDs was 1:2 495 live born infants.

As mentioned, ENBS was begun in June 2012 as a pilot study covering the total newborn population in Slovakia, and on 1 January 2013, it was introduced as an obligatory method provided to all newborns in Slovakia and covered by health insurance. During this pilot study provided in 28 000 newborns, 12 IMDs were detected, 8 PKU/HPA, 1 leucinos (MSUD) and 3 MCAD.

The results of complete ENBS in Slovakia (1 June 2012 – 31 December 2013) are presented in Table 2. During one-and-a-half years, 82 893 newborns were investigated and 34 cases of IMDs were detected. A screening prevalence of IMDs of 1:2438 live born infants was confirmed. Excluding 24 IMDs cases in the regular ENBS (PKU/HPA, MCAD, MSUD – incidence 1:3 454), an additional 10 cases at the perimeter of the method were detected with a prevalence of 1:8 289 newborns. Except IMDs, 23 cases of CH (incidence 1:2 386), 4 cases of CAH (incidence 1:13 723) and 5 cases of CF (incidence 1:10 978) were detected in NBSC in 2013 (Table 1).

DISCUSSION AND CONCLUSION

The presented results and experiences with ENBS in Slovakia are interesting from a couple of aspects. For one, as mentioned earlier, the LC-MS/MS method from DBS detects even 73 analytes en block in one DBS sample. Some of them are possible markers of various IMDs, evaluated by an online system and a special software program. Officially in Slovakia, the 10 most recommended IMDs were included in regular ENBS (PKU, MSUD, GAI, IVA, MCAD, LCHAD, SCAD, CPTI, CPT II, CACT) (Dluholucký, Knapková and Záhorcová 2013). This

regular ENBS was officially defined in the guideline of Slovak Ministry of Health, and financially covered through health insurance, and provided accordingly routine NBS algorithm (Professional Guidance of the Ministry of health of the Slovak republic 42/2012).

But, the method of LC-MS/MS detects many other deviations at the perimeter (Wilcken et al., 2001, Lam et al., 2013). This allows expansion of NBS and many other IMDs, which are detected during regular ENBS in various countries in Europe (Loeber et al. 2012). In Slovak ENBS, these additional suspect IMDs are carefully and individually evaluated and resolved until the definitive diagnosis and/or its negativity is provided. As presented in our results, the clarification of the perimeter of the method represents almost 29% of all cases (10 from 34 cases). An interesting case was galactosemia detected per unusually high phenylalanine and tyrosine levels using LC-MS/MS screening method. A complete examination of this case revealed the classical form of galactosemia. Similarly interesting are the cases of maternal inherited deficits, 3 methyl crotonyl CoA deficiency (3-MCCD) and CUD, detected through NBS of the newborns (Schimmenti et al. 2007). They are in adult medical care and the infants are in good health. After our one-and-a-half year experience, we can state that ENBS by means of LC-MS/MS provides the opportunity for detection of a wide spectrum of IMDs, even those not recommended or included in regular ENBS for various reasons (not fulfilling W-J criteria, economic aspect, etc.). Whilst the total prevalence of IMDs in our ENBS is 1:3 454, the prevalence of the IMDs at the perimeter is 1:8 289, which is comparable with the incidence of PKU or CAH (diseases included in regular NBS).

After our preliminary results of ENBS in Slovakia, we conclude that the introduction of LC-MS/MS method into NBS significantly expands the early detection possibilities.

Expanded screening methods allow early treatment and thus improve the quality of life; however, they are important also from a scientific point of view as research improvements. Nevertheless, high quality screening provides high quality data about the prevalence of disease in the country and thus allows reasonable financial planning in the health system.

Table 2. Results of expanded newborn screening (ENBS) tandem mass spectrometry ENBS 1 June 2012 – 31 December 2013 (82 893 newborns)

Disorder	No. of positive cases	Prevalence
Total	34	1:2 438
PKU/HPA	17	1:4 876
MCAD	6	1:13 815
Other: Propionic acidemia (1), MSUD (1), hyperthyrozinemia (2), 3-MCC (2), galactosemia (1), maternal CUD (2), maternal 3-MCC (2)	11	1:7 536

PKU/HPA phenylketonuria/hyperphenylalaninemia; MCAD medium chain acyl CoA dehydrogenase deficiency; CUD carnitine uptake deficiency; 3-MCCD 3-methylcrotonyl CoA carboxylase deficiency

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